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THE ANTI-VITAMINS

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THE ANTI-VITAMINS

By RUTH WOODS

Nearly half of the last century was spent by scientists all over the world anxiously seeking the cause of a dread disease which frequently killed off entire populations. The disease was pellagra — literally "rough skin," so named because of one of its major early symptoms. Those who survived it were described as "pitiable, half-mad wrecks of humanity."

The years spent in tracking down the cause of pellagra were marked by bitter and violent quarrels among scientists and even among political groups. The controversy centered on this question: Was a germ or a poison in food the cause of the disease, or was it the *absence* of something vital in food? The concept of a "deficiency disease" had not yet been fully evolved; Pasteur's germ theory was still excitingly new. So, it is understandable that the idea of a *negative cause* of a disease was extremely difficult to grasp.

The spectacular studies of Goldberger finally demonstrated that pellagra was indeed a "deficiency disease." Soon afterward the deficient food ingredient was found to be nicotinic acid, a new vitamin. The chapter on pellagra was closed; the "poison in corn" theory shamefully discredited. But not for long! One puzzling fact still remained. People in corn-growing regions were dying of pellagra — even with nicotinic acid in their food! Other people, whose food contained less nicotinic acid but who were not forced to subsist mainly on corn, were surprisingly free of pellagra. Something was seriously amiss in this situation. Not until six years ago was the mystery solved.

Strangely enough it was research in an entirely new field—chemotherapy —which provided a means of reconciling the puzzling discrepancies in the pellagra picture. The "deficiency" nature of pellagra was upheld, but so in a way was the corn "toxin" theory: The existence of anti-vitamins had been discovered.

The discovery of the anti-vitamins (also anti-hormones and other anti-metabolites) is of considerable significance. First it has brought to light a new concept—*the fact that certain compounds almost identical in structure with various essential metabolites are able to induce in animals many of the deficiency signs associated with a lack of these metabolites.* Second, investigation into the mechanism of this antagonistic behavior has provided a new tool for understanding important biological actions and may eventually lead to a clearer understanding of how vitamins* work in the body. Third, the discovery of anti-vitamins has practical applications in therapy as well as in nutrition research. Fourth, the dietary importance of certain foods may require reevaluation because of their possible anti-vitamin content.

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*This applies, of course, to enzymes, hormones, protein derivatives and other important metabolites as well.

Evolution of the Anti-Vitamin Concept:

The story of the anti-vitamins does not go back to a single piece of research for its beginning. Rather, it has been pieced together like a jig-saw puzzle from clues scattered over many years in extremely divergent fields of scientific investigation. The pellagra mystery provided one of these clues; the discovery of a dramatic new therapeutic agent, sulfanilamide, provided another. An examination of these clues and of how they were integrated to form a single new concept constitutes an exciting chapter in the annals of science.

Following the identification of nicotinic acid as the pellagra-preventive factor in 1937 by Elvehjem, Madden, Strong and Woolley (1), these investigators tested a series of chemicals related to nicotinic acid for their relative vitamin potencies. In his way it was hoped that some basis might be acquired for determining the relationship between structure and vitamin activity (2). One of these related chemicals, 3-acetylpyridine (Fig. 1), was found to have no vitamin activity, but amazingly it was shown to be actually poisonous to nicotinic acid-deficient animals. Normal animals were unharmed by the compound. There seemed to be no explanation for the peculiar behavior of this compound at the time.

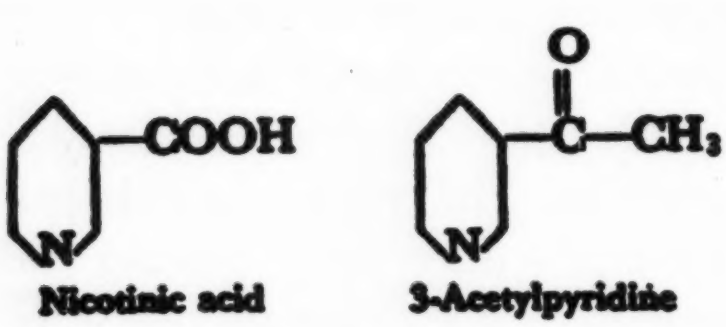

FIG. 1

Then in 1940, D. D. Woods (3) demonstrated the surprising fact that the power of the sulfonamides to interfere with bacterial growth could be completely overcome by adding a structurally related microbial growth factor, *p*-aminobenzoic acid, to the bacterial culture media (Fig. 2). Woods' purpose in using *p*-aminobenzoic acid was, like that of Elvehjem's group, to determine the relative bacteriostatic potencies of structurally related compounds. Again, like the analogue of nicotinic acid, the analogue of sulfanilamide produced a result exactly the reverse of what was expected. The theory was set forth that sulfanilamide owed its bacteriostatic activity to the fact that it prevented bacteria from using the essential growth factor, *p*-aminobenzoic acid.

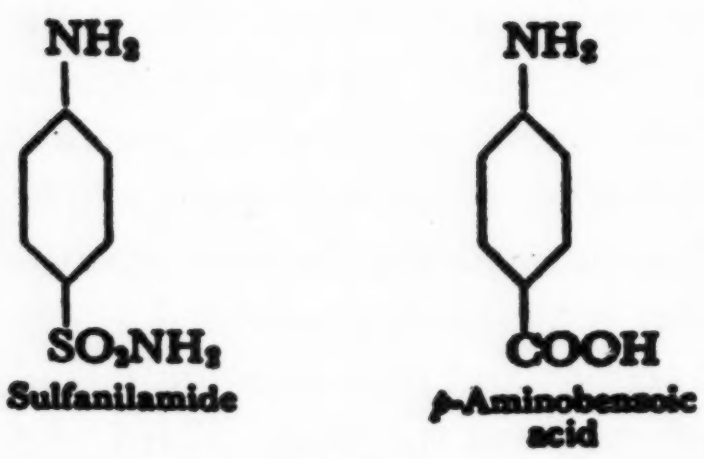

FIG. 2

Conversely, providing extra *p*-aminobenzoic acid to bacteria which had been paralyzed by sulfonamide enabled these disease-producing microorgan-

isms to resume their normal growth. This competitive action between the sulfa drug and the microbial growth factor was believed to be possible because of the closely related structures of the two compounds.

Theories similar to this had been suggested before with respect to other groups of related compounds, but little attention had been paid to them. When the peculiar competitive behavior between metabolites and structurally related compounds turned out to be involved in the story of the newly discovered "miracle" drugs, the sulfonamides, a lively interest in the phenomenon sprang up. The possibility presented itself that new anti-bacterial drugs might be almost "custom-tailored" by creating compounds similar in structure, but antagonistic in action, to the various growth factors essential for disease microbes.

Anti-Vitamins as Chemotherapeutic Agents:

Some real successes along these lines were soon reported. A year after Woods' work with sulfanilamide and *p*-aminobenzoic acid, Fildes (4) demonstrated that a simple alteration of the tryptophane molecule to yield indole acrylic acid resulted in the production of an antibacterial agent. In the meantime, coincident with the earlier work on *p*-aminobenzoic acid, McIlwain (5) made another interesting discovery. He found that the same structural change involved in transforming the nutrient *p*-aminobenzoic acid to sulfanilamide, if applied to the nutrient nicotinic acid, would produce another bacteriostatic agent, pyridine-3-sulfonic acid. In each case, the carboxyl group (COOH) of the original nutrient had been replaced by a sulfonic acid (SO_3H) or amide (SO_2NH_2) group. Snell (6) provided still another example of two competitive, mutually inhibiting compounds by using this same procedure to replace the carboxyl group of pantothenic acid with sulfonic acid, thereby producing thiopanic acid. The chart on page 4 illustrates the changes made in the metabolites, *p*-aminobenzoic acid, nicotinic acid and pantothenic acid to yield the corresponding anti-metabolites.

It is interesting to note that this type of structural change in the vitamin molecule produces an anti-vitamin which causes deficiency symptoms in bacteria (i.e. growth inhibition) but not in the higher animals.* Therefore, this type of anti-vitamin has been found useful in treating infectious diseases—the antagonistic effects are exerted on the disease-producing microorganisms without disturbing the normal metabolism of the host animal.

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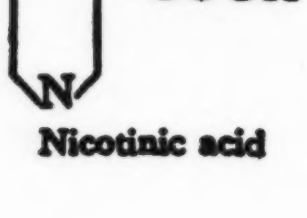
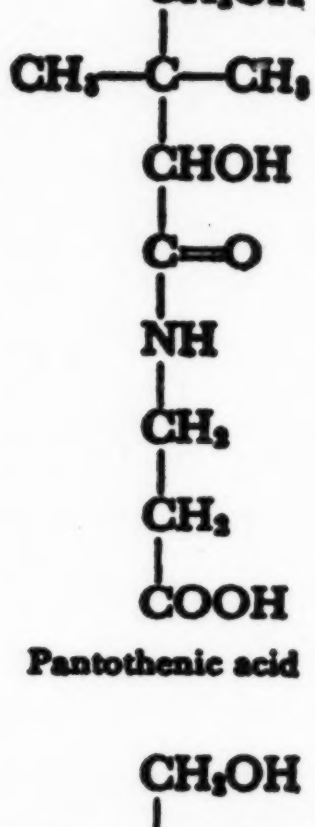
*An exception to this is the sulfonic acid analogue of nicotinic acid. This, as well as 3-acetylpyridine (the nicotinic acid analogue discussed earlier in this *Review)* has been shown to produce symptoms of nicotinic acid deficiency in dogs.

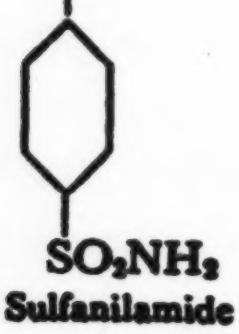
p-Aminobenzoic acid



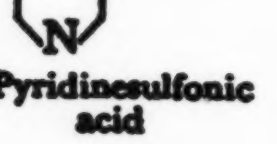
Nicotinic acid



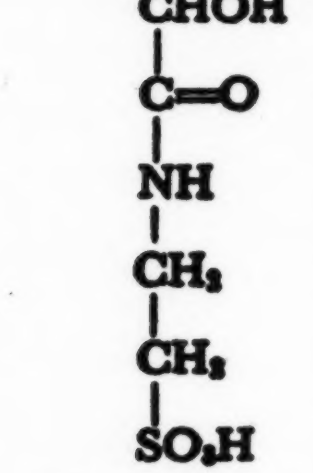
Pantothenic acid



Sulfanilamide



3-Pyridinesulfonic acid



Thiopanic acid

FIG. 3

Still other inhibitory analogues of these three vitamins may be produced by substituting a ketone grouping (-COR) for the carboxyl group (COOH), yielding anti-metabolites, aminoacetophenone, 3-acetylpyridine and phenylpantothenone, for *p*-aminobenzoic acid, nicotinic acid and pantothenic acid, respectively.

Anti-Vitamins in Animals:

Although the sulfonamides had provided a starting point for the new field of anti-metabolites, research workers soon began to wonder whether the scope of such antagonists might not exceed the realm of microbes and extend into the demain of higher organisms. The search for animal anti-vitamins was on. True to expectations a series of compounds was soon discovered which were capable of calling forth in animals the signs characteristically associated with specific vitamin deficiencies.

Before looking into the provocative question as to why scientists should want to produce the very deficiencies they have spent lifetimes on preventing and curing, it might be interesting to first examine the results of these strange efforts.

Thiamine and Pyrithiamine: Among the first of the animal anti-vitamins to be created was pyrithiamine, prepared in 1943 by Woolley and White (7). As its name indicates, this compound antagonizes the action of thiamine (Fig. 4). When fed to mice pyrithiamin, a pyridine analogue of the vitamin, produces typical deficiency signs within a few days. The animals become unable to stand upright on their hind legs, toppling over backward when attempting to do so. They rapidly become overly irritable, lose their appetites and finally fall into convulsions, either without any provocation at all or especially when picked up by the tail. Other more serious signs of progressive thiamine deficiency appear, leading eventually to death. Woolley *et al* found that increasing the amount of thiamine in the diet protected the animals from the effects of pyrithiamine. Furthermore, when the animals once had developed thiamine deficiency symptoms due to the administration of pyrithiamine, it was possible to cure them, even in the terminal stages of the disease, by administering thiamine. Woolley and White (8) made the additional interesting observation that pyrithiamine is also an active antibacterial agent and that its effects can be counteracted by increasing the supply of thiamine in the culture medium.

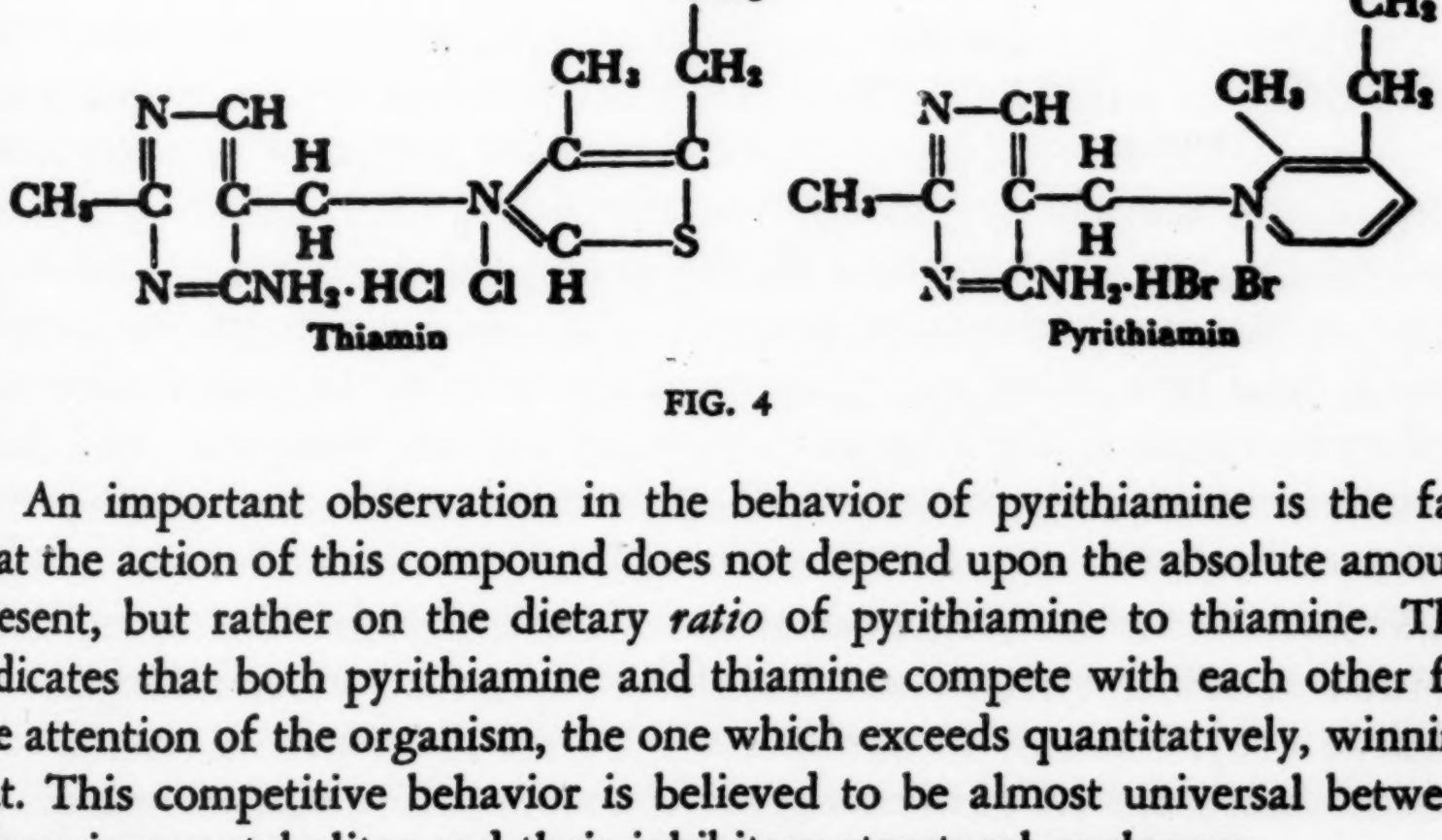


FIG. 4

An important observation in the behavior of pyrithiamine is the fact that the action of this compound does not depend upon the absolute amount present, but rather on the dietary *ratio* of pyrithiamine to thiamine. This indicates that both pyrithiamine and thiamine compete with each other for the attention of the organism, the one which exceeds quantitatively, winning out. This competitive behavior is believed to be almost universal between the various metabolites and their inhibitory structural analogues.

Following the studies with pyrithiamine, analogues of other vitamins were produced which were found to cause diseases characteristic of those occurring with a deficiency of the related vitamin. These diseases were believed to be really deficiencies not only because they presented similar symptoms and disturbances, but also because they were prevented or cured by adequate amounts of the vitamins concerned.

Ascorbic Acid and Glucoascorbic Acid: Experiments with the inhibitory analogue of vitamin C were particularly remarkable because the subjects of the tests, mice, have never been known to develop scurvy. Mice do not need a dietary supply of ascorbic acid, apparently meeting their requirements for this vitamin by internal synthesis. Consequently a scorbutic mouse had never been seen until glucoascorbic acid was fed to a group of these animals (9). Every one of these soon came down with many of the signs of scurvy as usually seen in guinea pigs or other animals susceptible to the disease. What had occurred was obviously a competitive struggle between the internally synthesized vitamin and glucoascorbic acid for supremacy in the biological system. As was expected, glucoascorbic acid of course also precipitated symptoms of scurvy in those animals who ordinarily require vitamin C in the diet. In these animals, the deficiency was promptly corrected by ascorbic acid (9).

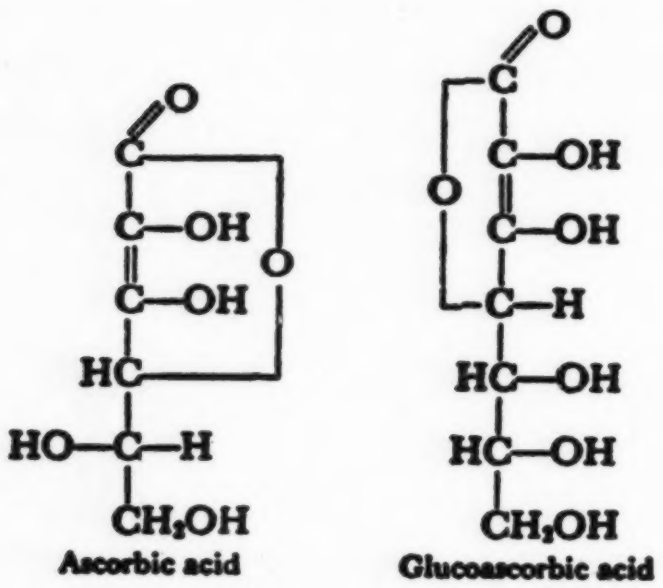


FIG. 5

Other B Vitamins and Inhibitors: Continued studies have established an antagonistic relationship between riboflavin and any one of three structurally related compounds (10). Nicotinic acid deficiency, like scurvy, never encountered in mice, was induced by feeding 3-acetylpyridine (11), recalling and explaining the first experiments with this compound (see page 2). It is interesting to note that another nicotinic acid analogue, 3-pyridine-sulfonic acid, is fatal to nicotinic-acid deficient dogs, harmless to normal dogs and ineffective in mice. Phenylpantothenone as well as thiopanic acid, both previously mentioned, compete with pantothenic acid; desthiobiotin with biotin, and so on.

Vitamin K, Vitamin E and Inhibitors: Dicoumarol, first discovered as the injurious constituent of spoiled sweet clover hay causing vitamin K deficiency in cattle (12), was only in retrospect discovered to be an analogue of vitamin K. Similarly, 2,4-dichloro-naphthoquinone, a powerful fungicide developed empirically during the war (13), was later recognized as an analogue

of vitamin K. As might have been predicted, the microbial growth-inhibiting powers of this compound were reversed when vitamin K was provided (14). Strangely related to both vitamin K (which is 2-methyl-1, 4-naphthoquinone) and to vitamin E (which is α-tocopherol) is the compound α-tocopherol quinone. This compound produces signs of vitamin E deficiency in rats and mice, but its effects are counteracted by vitamin K instead of vitamin E! (15).

Significance of Anti-Vitamins:

As Therapeutic Agents: We may now go back to the question: Why produce vitamin antagonists? Or, for that matter, anti-hormones, anti-amino acids and the like? As has already been mentioned, because the phenomenon of competitive antagonism was first popularized by the sulfonamide-*p*-amino-benzoic acid relationship, the belief arose that other chemotherapeutic agents might be developed against infectious diseases by producing analogues of metabolites. Some of these have already been discussed. It is interesting to note that the most practical of these, 2,4-dichloronaphthoquinone, was not recognized as a competitive analogue of vitamin K until after it had been developed by other standards. The sulfonic acid analogue of pantothenic acid, thiopanic acid, was shown to be capable of protecting rats from streptococcal infection (16). But its action is too weak for it to be of practical therapeutic importance. However, the possibility of finding useful therapeutic agents by this means is increasing with continued research.

Recent reports (17) have described some highly interesting experiments designed to develop an inhibitory analogue useful against infection by certain intracellular micro-organisms. At present there are no therapeutic means of control for such micro-organisms as there are for bacteria. Thus while streptococcal or pneumococcal infections may yield to penicillin or the sulfonamides, there are no effective agents for poliomyelitis or influenza.

Although no such agent for these two diseases has yet been found, Woolley's group has reported a substance which will work against intracellular parasites. This is phenyl pantothenone, an inhibitory structural analogue of pantothenic acid. It has been found to be an active antimalarial agent. Phenyl pantothenone is about as active as quinine, while some of its derivatives are even more active. Since much of this work still remains shrouded under military secrecy, there are few details available at present concerning this fascinating development beyond the fact that this compound was originally tested because of observations indicating the importance of pantothenic acid in parasite metabolism.

The applications of anti-metabolites may lead to still other types of therapeutic agents besides those used to combat infectious agents. For example,

it is quite possible that, under certain circumstances, it may become desirable to produce for therapeutic purposes symptoms of a vitamin or hormone deficiency. Why deliberately produce a deficiency state when everyone is trying to prevent this with vitamin pills and hormone injections? Woolley cites two interesting answers to this question:

First is the well-known story of dicoumarol. This compound was found to produce hemorrhages characteristic of vitamin K deficiency (18). At first, dicoumarol was the bane of the farmer's existence, leading to disastrous consequences for the cattle which fed upon the spoiled sweet clover hay containing it. When it was discovered that vitamin K would cure the animals of the symptoms induced by dicoumarol, the realization also came that the two compounds are structural analogues. Dicoumarol is now used clinically before operations in order to prevent dangerous blood clots from forming.

Knowing both the effects of vitamins and of their absence on bodily function provides potentially a spectacular means of controlling at will physiological responses by administering vitamins (as is now done) or their structurally related inhibitors.

With this precept in mind, Woolley (19) set out to produce a selective pharmacological agent which would cause a response the nature of which could be predicted even before the compound was synthesized and which had never before been caused by any known drug.

The metabolite selected for this experiment was tocopherol, vitamin E. It was chosen because in mice a deficiency of this vitamin causes a highly specific response only in the pregnant individual. It is not fatal to the mother animal and is without effect on the non-pregnant female mouse.* Vitamin E deficiency in the pregnant mouse causes a resorption of the embryo during the latter part of gestation. This terminates the pregnancy and removes all

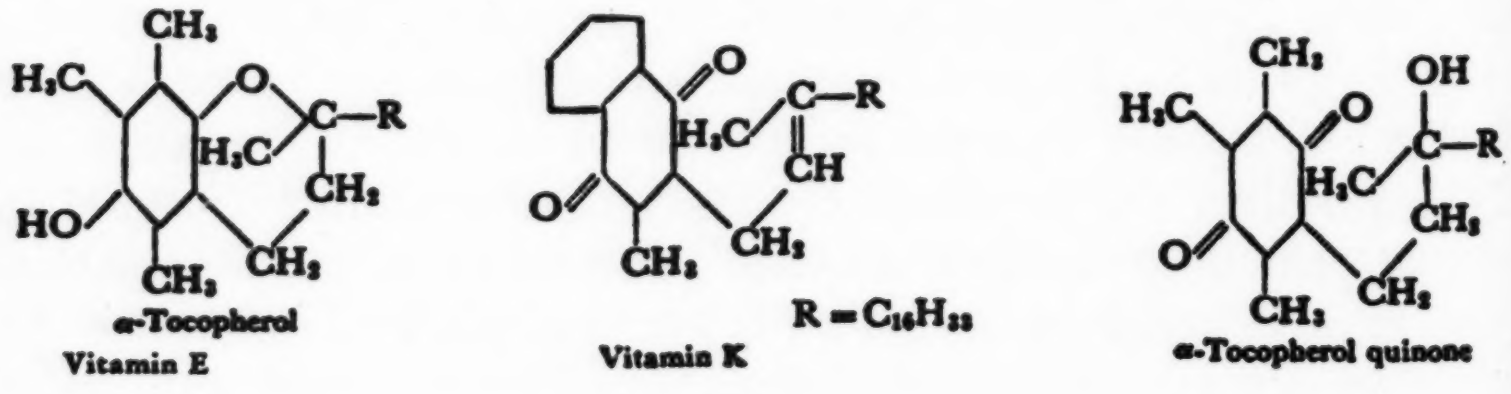


FIG. 6

traces of the embryo. A successful antagonist of tocopherol would, therefore, be expected to produce such a resorptive interruption of pregnancy in mice. Just such an analogue was found in α-tocopherol quinone (19). Curiously

*cf.—*Borden's Review of Nutrition Research,* June 1945, "Experimental Vitamin E Deficiency," and September 1945, "Vitamin E in Veterinary and Clinical Medicine."

enough, as can be noted from Figure 6, this compound is related both to vitamin E and to vitamin K. When α-tocopherol quinone was fed to pregnant mice the desired pharmacological effect—termination of pregnancy—was obtained. Perhaps the strangest part of the experiment was the fact that although the effect obtained was characteristic of vitamin E deficiency, vitamin K *and not vitamin E* was the only means of reversing the action of this compound. This study although not clinically practical because of the tremendous doses of α-tocopherol quinone required, serves, however, as an experimental model to point the way to new and useful avenues of attack in the employment of structural anti-vitamins.

As Food Factors: Anti-vitamins may occur naturally in certain foods. This brings up the problem that certain foods may actually nullify the vitamin content of other foods and lead to an over-all increase in the vitamin intake requirement.

As an important example of this, we refer again to the history of pellagra. By 1937, nicotinic acid had been clearly established as the pellagra-preventive vitamin. Nevertheless, the "corn-toxin" theory could not be completely discarded, particularly in the face of new evidence from India where pellagra is an urgent problem. From that country Aykroyd and Swaminathan (20) reported that people whose diets contained corn and also supplied 15 milligrams of nicotinic acid per day were succumbing to pellagra. However, other persons receiving only 5 milligrams of nicotinic acid per day in a corn-free diet never developed the disease!* That these observations were no mere accident was shown when, five years later, investigators in this country (21) were able to inhibit the growth of rats by feeding them corn. Normal growth was resumed as soon as extra nicotinic acid was provided. A suggestion was made that interference with intestinal vitamin synthesis might be the cause, but there was no evidence to support the theory.

What seems at last to be the real explanation of the mystery was described by Woolley (22) only a few months ago. Recalling the early studies with 3-acetylpyridine as a cause of pellagra in mice (2), he suggested that "corn contains a structural analogue of nicotinic acid which acts as a *positive* etiological factor in pellagra." In support of this theory it was next demonstrated that such a substance could be extracted from corn and that, when fed to mice, it would cause pellagra. The effect of this as yet unidentified corn extract† is counteracted by nicotinic acid. According to Woolley:

> *"Pellagra is a deficiency disease which results partly from a lack of sufficient nicotinic acid, but more especially from the action of an antagonistic agent in corn which competes with nicotinic acid and thereby intensifies the deficiency."* (17).

*The recommended daily allowance for nicotinic acid is from 15 to 18 mgs.
†Apparently a pyridine compound.

Theoretical Considerations:

The theory of competitive inhibition of compounds by their structural analogues is not only a relatively new one, but it is still largely in the formative stage. While most of the mechanics of pure chemistry and enzymology which are involved in structural antagonisms are beyond the scope of this paper, it seems desirable from the point of view of clarity and completeness to highlight a few of these theoretical principles.

What is Competitive Inhibition? It has already been pointed out that the antagonistic behavior of structurally similar compounds is usually the result of such compounds competing for the attention of the organism. The most likely theory to date—but one which is not entirely free of flaws or exceptions—states that the analogue competes with the vitamin for a specific site in an enzyme (23, 24, 3, 25). Either compound can occupy this site at the expense of the other since both, being closely alike in structure, can react with the chemical groups of the enzyme. If the vitamin combines with the enzyme group, the compound formed passes normally through the metabolic reactions for which the system is adapted. If the analogue of the vitamin combines with the enzyme group, a foreign compound is formed which does not fit into the biological scheme and is therefore valueless to the body.

In this way, the analogue prevents the body from making use of the vitamin-enzyme system, thereby calling forth the same symptoms as would occur in an actual deficiency of the vitamin. Whether the vitamin or its analogue combines with the enzyme depends on the relative concentrations of the two. Thus, it is not the presence of the analogue *per se* which will inhibit the action of the corresponding vitamin, but rather the ratio of analogue to vitamin which will determine the course of action.

The phenomenon of competitive inhibition has a number of interesting phases. For example, the action of inhibitory analogues varies with the animal species tested being inhibitory in some and not in others. Also, in some cases the related vitamin will not reverse the action of the inhibitor. Again, in some species, the analogue will be inhibitory only if the corresponding vitamin is a nutritive essential for the particular species. In other animals, no such correlation exists.

A final point of interest is the fact that antagonisms of a non-competitive type have also been brought to light. These antagonisms have nothing to do with structural similarities between the compounds involved and present an entirely different subject in themselves. In this class of antagonisms the inhibitors are generally proteins or amino acids which work by directly inactivating the vitamins (or other metabolites) involved. Examples of these include ascorbic acid oxidase which destroys ascorbic acid by hastening its

oxidation; avidin, the mysterious constituent of egg white, which combines with biotin thereby rendering it unavailable; and an unidentified enzyme in raw fish which destroys thiamine by splitting it into fragments which the body cannot make use of. Two other interesting examples are the reversal of the action of acetylpyridine (the anti-nicotinic acid analogue) by the amino acid tryptophane, and the reversal of the action of the sulfonamides (anti-*p*-aminobenzoic acid analogue) by the amino acid methionine.

Conclusion:

The discovery of the anti-vitamins marks a new chapter in the history of nutrition research. The early study of vitamins established the principle that certain compounds present in trace quantities in the diet had vital *positive* biological functions. The studies outlined in this *Review* have now shown that other compounds may have equal significance in a *negative* direction. These latter compounds exert their effects by interfering with the action of the vitamins themselves. This means that from the point of view of nutrition, one must not think in terms of *absolute* vitamin intake or requirements, but in terms of *net* vitamin availability.

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